

# INCIDENCE OF SPINAL DEFORMITIES IN NATURAL POPULATIONS OF GRASS GOBY, *ZOSTERISESSOR OPHIOCEPHALUS* FROM THE KARIN SEA, EASTERN MIDDLE ADRIATIC

by

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**ABSTRACT.** - Information is presented on the occurrence of spinal deformities in natural populations of grass goby, *Zosterisessor ophiocephalus* (Pallas, 1811) in the Karin Sea, Eastern Middle Adriatic (44°09'N-15°37'E). During 2002, the spinal deformity levels varied between 8.6% (in March) and 20.9% (in May), and 16.5% in total catch. The spinal column is deformed in one to few places from vertical to horizontal angles, and the deformities are visible on the fish body immediately after catching. The possible reasons for such deformities are discussed.

**RÉSUMÉ.** - Incidence des déformations des vertèbres dans les populations naturelles de *Zosterisessor ophiocephalus* de la mer de Karin, Est Adriatique.

Des informations sont présentées sur la présence de déformations des vertèbres chez des populations naturelles du gobie, *Zosterisessor ophiocephalus* (Pallas, 1811) dans la mer de Karin, dans l'est de l'Adriatique (44°09'N-15°37'E). En 2002, le pourcentage des déformations variait entre 8,6% (en mars) et 20,9% en mai, et était de 16,5% pour l'ensemble des captures. La colonne vertébrale est déformée en un ou quelques endroits selon des angles verticaux et horizontaux, et ces déformations sont visibles sur le corps du poisson immédiatement après sa capture. Les possibles raisons de ces déformations sont discutées.

Key words. - Gobiidae - *Zosterisessor ophiocephalus* - MED - Karin Sea - Adriatic - Natural populations - Spinal deformities.

Spinal deformities in natural and reared fish populations are relatively well described and many reports have been published on different fish species (Slooff, 1982; Endo and Iwatsuki, 1998). Capture of deformed fish in more or less polluted environments is common (Slooff, 1982; Antunes and Lopes da Cunha, 2002). If the level of deformities is high and constant and exists in different age classes, it could be a sign of significant ecosystem changes or genetic changes of population. This could influence the fishery if deformities exist in populations of commercially important species.

The findings of deformed fish in the Adriatic are not rare, but they are limited to sporadically caught specimens (Morović, 1954; Jardas and Morović, 1973; Jardas and Homen, 1977). The higher frequency of spinal deformities was reported only for red mullet, *Mullus barbatus* but without detailed analysis (Jardas and Morović, 1975), and for sandsmelt, *Atherina boyeri* with discussion on the possible reasons for such deformities (Tutman *et al.*, 2000).

Grass goby, *Zosterisessor ophiocephalus* (Pallas, 1811) (Gobiidae) is a species that lives in the inshore and brackish water of estuaries and lagoons of the Mediterranean, Black Sea, Adriatic Sea and Sea of Azov (Jardas, 1996). In the Adriatic, especially on the Eastern coast, it has some local economical importance, mostly living and reproducing in

coastal waters and estuaries during its whole life cycle (sexually mature at two or three years; lifespan is five years) (Jardas, 1996). It is exposed to many physical and chemical variations, from temperature and salinity changes to pollution, in these most threatened ecosystems.

This paper describes the spinal deformities found in natural populations of grass goby, *Zosterisessor ophiocephalus* in the Karin Sea, Eastern Middle Adriatic.

## MATERIAL AND METHODS

Fish were caught with a small trawl (called "tartana") during January, February, March, April and May 2002 in the Karin Sea, situated on the Mid-East Adriatic coast (44°09'N-15°37'E) (Fig. 1). This area is under a great influence of the River Zrmanja.

Caught fish were counted and the percentage of deformation was calculated. A total of 2618 fish was caught (489 in January, 478 in February, 706 in March, 528 in April and 417 in May) and 358 were deformed, which represents 16.5% of the total. The total length (mm) and weight (g) of fresh specimens were measured (Fig. 2). After that specimens were frozen and transported to the laboratory where

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Table I. - Internal and external abnormality ranking system for grass goby from Karin Sea (Adriatic) (adapted from Goede and Barton, 1990, and Herman, 1990).

| Feature                                 | Ranks          |                  |                    |                               |
|-----------------------------------------|----------------|------------------|--------------------|-------------------------------|
|                                         | 1              | 2                | 3                  | 4                             |
| Spinal: (kyphosis, lordosis, scoliosis) | No abnormality | Slight curvature | Moderate curvature | Significant-extreme curvature |

they were sexed and dissected for the detection of deformities. From the total examined, 1069 were males ranging between 81 and 169 mm total length and 1549 females between 83 and 171 mm. Age was determined by otolith reading.

Key skeletal anomalies were: 1) kyphosis, typically a convex curvature of the spinal column in the thoracic region creating a «humpback» appearance; 2) lordosis, a concave curvature of the spinal column in the lumbar region; 3) scoliosis, abnormal lateral curvature of the spinal column. The system used to rank anomalies was adapted from Goede and Barton (1990) and Herman (1990) (Tab. I). The condition factor (IC) of normal and deformed fish was calculated using the formula:  $IC = 100W/L^3$  (W is weight, L is total length) and the difference was calculated using a t-test. The fresh specimens (lateral aspect) were radiographed using a medical X-ray system and the radiographs were used to inspect vertebrae and caudal bones, and to evaluate anomalies observed in specimens.

Data were entered into Microsoft Excel 97 SR-2 spreadsheets and statistical analysis was conducted using SPSS v7.5.3. and Microsoft Excel 97 SR-2. The nonparametric Kruskal-Wallis ANOVA and the Scheffe comparison tests were used to determine the significance of anomaly rates through time (using month), between sexes, and by age to

evaluate whether the anomalies were more prevalent in younger, smaller fish, indicating possible genetic origin, or whether the anomalies were developed later in life, indicating potential environmental influences.

## RESULTS

Spinal anomalies were visible on the fish body immediately after capture, with the spine curved at one or more places. The internal body spaces and organs were normally developed, according to X-ray radiographs (Fig. 2). Vertebral deformities were noticed, especially at curvation points. The vertebra (centrum) was considerably thicker and shorter than normal (normal specimens: mean length =  $3.2 \text{ mm} \pm 0.19$ ; deformed specimens: mean length =  $1.9 \text{ mm} \pm 0.28$ ), particularly at the ends, and the intervertebral ligament were much thicker than normal. Sometimes vertebrae were shortened but not thickened as if two vertebrae occupied the

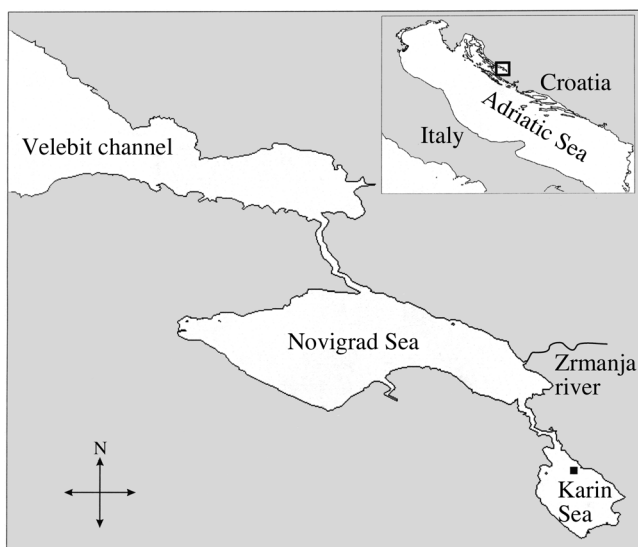


Figure 1. - Sampling station of grass goby in the Karin Sea, Eastern Middle Adriatic.

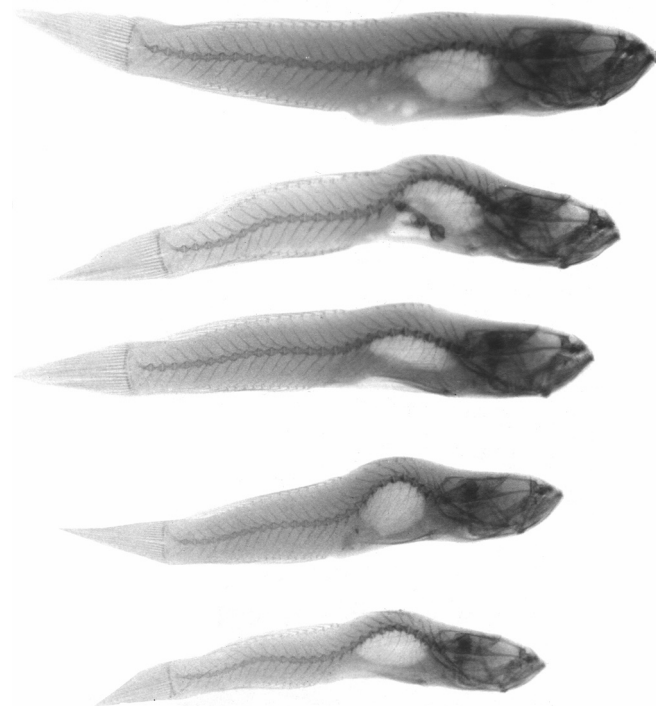


Figure 2. - X-ray radiographs of deformed spines of grass goby from the Karin Sea, Eastern Middle Adriatic.

space of a normal one. Very seldom deformed vertebrae induced column curvature. There were no visible deformities of the remaining skeletal parts (skull, fin, tail bones).

The percent of deformed fish in monthly catches was 14.1% in January, 11.1% in February, 8.6% in March, 16.7% in April and 20.9% in May (Tab. II) and the difference was significant ( $p < 0.05$ ) between some months (May-March, May-February, April-March). The total length of deformed specimens ranged from 8.1 to 17.1 cm, and the weight from 5.21 to 41.56 g. Vertebral deformities were observed in 358 specimens corresponding to 16.5% of the total examined (217 males, 141 females). Males were more deformed than females, 8.3% against 5.4%, respectively, and difference was significant ( $p < 0.05$ ). The highest percent of deformities was observed in May (20.9%), while lowest in March (8.6%) (Tab. II). According to temporal distribution of spinal deformities between males and females it was observed that significantly higher percent ( $p < 0.05$ ) of males dominated during all months. Combination of two anomalies were found in 37 specimens (10.3%) (kyphosis-lordosis: 17 specimens; kyphosis-scoliosis: 8 specimens; lordosis-scoliosis: 12 specimens), while only 5 specimens (1.4%) showed combination of all anomalies.

Of the anomaly rates differing significantly among months and sexes, scoliosis was the highest (52%) (Tab. III). The highest percent of scoliosis and kyphosis was found in May, while of lordosis in January. Most anomalies were ranked as significant-extreme curvature (rank 4) (Tab. IV).

The spinal deformities occurred in different year classes

Table II. - Monthly distribution of grass goby with spinal deformities in Karin Sea. n = number of fish examined; s = number of fish with spinal deformities.

| Month    | Males |     |      | Females |     |      | Total |     |      |
|----------|-------|-----|------|---------|-----|------|-------|-----|------|
|          | n     | s   | %    | n       | s   | %    | n     | s   | %    |
| January  | 227   | 40  | 17.6 | 262     | 29  | 11.1 | 489   | 69  | 14.1 |
| February | 166   | 37  | 22.3 | 312     | 16  | 5.1  | 478   | 53  | 11.1 |
| March    | 315   | 39  | 12.4 | 391     | 22  | 5.6  | 706   | 61  | 8.6  |
| April    | 229   | 49  | 21.4 | 299     | 39  | 13.0 | 528   | 88  | 16.7 |
| May      | 132   | 50  | 37.9 | 285     | 37  | 13.0 | 417   | 87  | 20.9 |
| Total    | 1069  | 215 | 20.1 | 1549    | 143 | 9.2  | 2618  | 358 | 16.5 |

| Month | Feature: spinal |      |    |    |          |      |    |    |           |      |     |    | Total |      |     |     |
|-------|-----------------|------|----|----|----------|------|----|----|-----------|------|-----|----|-------|------|-----|-----|
|       | Kyphosis        |      |    |    | Lordosis |      |    |    | Scoliosis |      |     |    |       |      |     |     |
|       | n               | %    | M  | F  | n        | %    | M  | F  | n         | %    | M   | F  | n     | %    | M   | F   |
| Jan   | 12              | 17.3 | 6  | 6  | 37       | 53.6 | 20 | 17 | 20        | 29   | 14  | 6  | 69    | 19.3 | 40  | 29  |
| Feb   | 8               | 15.1 | 6  | 2  | 19       | 35.8 | 17 | 2  | 26        | 49.1 | 14  | 12 | 53    | 14.8 | 37  | 16  |
| Mar   | 11              | 18.0 | 7  | 4  | 17       | 27.9 | 9  | 8  | 33        | 54.1 | 19  | 14 | 61    | 17.0 | 35  | 26  |
| Apr   | 5               | 8.7  | 4  | 1  | 31       | 35.2 | 21 | 10 | 52        | 59.1 | 29  | 23 | 88    | 24.6 | 54  | 34  |
| May   | 17              | 20.7 | 9  | 8  | 15       | 18.3 | 10 | 5  | 55        | 67.1 | 32  | 23 | 87    | 24.3 | 51  | 36  |
|       | 53              | 14.8 | 32 | 21 | 119      | 33.2 | 77 | 42 | 186       | 52.0 | 118 | 78 | 358   |      | 217 | 141 |

0+, 1+, 2+, and 3+ old fish (Tab. V). Higher percent of deformities occur in the younger specimens (both in males and females), whilst in older, deformities are lower and affect fewer vertebrae (Tab. V). The percentage decrease in older specimens suggests a differential mortality so that survival of deformed fish is impaired.

There is a noticeable slight decrease in the length and condition index of deformed grass goby in comparison to those normal, but the difference is not statistically significant ( $p > 0.05$ ). The condition index of normal grass goby varied from  $0.87 \pm 0.095$  in January to  $1.12 \pm 0.094$  in April, while of deformed from  $0.77 \pm 0.094$  in January to  $0.99 \pm 0.090$  in April.

Table IV. - Ranks of anomalies for grass goby in Karin Sea.

| Month    | Feature: spinal | Ranks |   |    |     |
|----------|-----------------|-------|---|----|-----|
|          |                 | 1     | 2 | 3  | 4   |
| January  | Kyphosis        | 405   | - | 4  | 8   |
|          | Lordosis        | 380   | 1 | 10 | 26  |
|          | Scoliosis       | 397   | - | 7  | 13  |
| February | Kyphosis        | 417   | - | 1  | 7   |
|          | Lordosis        | 406   | 1 | 5  | 13  |
|          | Scoliosis       | 399   | - | 10 | 16  |
| March    | Kyphosis        | 634   | - | 1  | 10  |
|          | Lordosis        | 628   | - | 4  | 13  |
|          | Scoliosis       | 617   | 1 | 6  | 26  |
| April    | Kyphosis        | 435   | - | -  | 5   |
|          | Lordosis        | 409   | - | 4  | 27  |
|          | Scoliosis       | 388   | 1 | 9  | 42  |
| May      | Kyphosis        | 313   | - | 3  | 14  |
|          | Lordosis        | 315   | - | 2  | 13  |
|          | Scoliosis       | 275   | 1 | 12 | 42  |
|          |                 |       | 5 | 78 | 275 |

| Age | Males | Females | Total |      |
|-----|-------|---------|-------|------|
|     |       |         | n     | %    |
| 0+  | 89    | 44      | 133   | 37.2 |
| 1+  | 63    | 39      | 102   | 28.5 |
| 2+  | 33    | 35      | 68    | 19.0 |
| 3+  | 30    | 23      | 53    | 14.8 |

Table V. - Anomaly rates for grass goby by age groups.

Table III. - Anomaly rates for grass goby by month and sex.

## DISCUSSION

Grossly anomalous fishes have been generally aware through angling, fishery and farming activities, or academic research, despite very low occurrence. In farmed fishes, the body shape anomalies and their pathogenesis have been reported by many researchers, because of their economic impacts on the production and marketing (Ferguson, 1989; Roberts and Bullock, 1989; Sindermann, 1990; Dedi *et al.*, 1995). On the other hand, there have been only a few reports on anomalous fishes from a view point of biological context, particularly in the Adriatic Sea (Jardas and Morović, 1975; Tutman *et al.*, 2000).

It was observed that the probability of capturing a deformed specimen depends on sex and size. Similar was found for *Gobius niger* from Sado estuary, Portugal (Antunes and Lopes da Cunha, 2002). Great differences in dimensions or sex ratio between samples could seriously impair the statistical significance of comparisons.

The appearance of spinal deformities in the different growth stages of grass goby in the Karin Sea suggests the existence of significant disturbances in the ecosystem. The fact that spinal deformities appear in 0+, 1+, 2+ and 3+ growth categories of grass goby suggests that ecosystem disturbances occur every year. The absence of older deformed grass goby in the population might be a sign that the disturbances are related to the last 3 or 4 years, but it could also be explained by higher mortality of deformed fish as compared to normal fish. Differences observed between males and females are probably related to the fact that ovaries are much heavier than testis so that swimming would be more difficult to grossly deformed females than to males, during reproduction season. Anomalies were more prominent in younger smaller fish indicating also possible genetic origin, but the question remains still open as to the causes of grass goby deformity in the Karin Sea. Vertebral deformities were more frequent in the 60/69 mm class of *Gobius niger* in Sado estuary (Portugal) decreasing thereafter with fish length, revealing an impact of fish survival (Antunes and Lopes da Cunha, 2002). There are some indices that such deformities occur on some other fish species and also on grass goby in the Novigrad Sea which is situated on the front of the Karin Sea (Baždarić, pers. comm.), and both seas are under the great influence of Zrmanja river estuary. As this was an metal industrial region (aluminium factory in the settlement Obrovac) contamination by metals or with some chemicals might be the cause. On the other hand, in some agricultural regions in vicinity of river Zrmanja, that uses pesticides and herbicides during spring and summer, i.e., during spawning and early development periods for grass goby, contamination by these chemicals might be also the cause. The study of deformity occurrences in fish seems to be a good and

practical way of assessing environment quality (Antunes and Lopes da Cunha, 2002). The conditions to such approach are: the target species abundance, easy capture and sensitivity to pollution without being eradicated (Slooff, 1982). A wide range of physical, chemical and biological factors may cause spinal deformities of various fish species in natural and reared conditions (Slooff, 1982; Haya, 1989; Weiss and Weiss, 1989; Chatain, 1994; Endo and Iwatsuki, 1998). Recent research on *Sparus aurata* indicates that most spinal deformities occur during embryonic development, and that the causes could be due to genetic, yolk content, and environmental conditions such as temperature, light, mechanical stress, and pollution, which could affect both parents and embryos (Andrades *et al.*, 1996). The factors inducing skeletal deformities under the breeding environment are presumed to be: shortage of nutrient substance, water temperature, concentration of  $\text{Ca}^{2+}$ , shortage of vitamin C, parasites, fish density and stress (Kitamura, 1969; Jardas and Homen, 1977). For this reason, further research of this phenomenon will concentrate on the spawning and early developmental stages of grass goby, but also on the accumulation of pollutants in the gonads of adult specimens.

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